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Relationships among Ciprofloxacin, Gatifloxacin, Levofloxacin, and Norfloxacin MICs for Fluoroquinolone-Resistant *Escherichia coli* Clinical Isolates[▽]

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Fluoroquinolones are some of the most prescribed antibiotics in the United States. Previously, we and others showed that the fluoroquinolones exhibit a class effect with regard to the CLSI-established breakpoints for resistance, such that decreased susceptibility (i.e., an increased MIC) to one fluoroquinolone means a simultaneously decreased susceptibility to all. For defined strains, however, clear differences exist in the pharmacodynamic properties of each fluoroquinolone and the extent to which resistance-associated genotypes affect the MICs of each fluoroquinolone. In a pilot study of 920 clinical *Escherichia coli* isolates, we uncovered tremendous variation in norfloxacin MICs. The MICs for all of the fluoroquinolone-resistant isolates exceeded the resistance breakpoint, reaching 1,000 μg/ml. Approximately 25% of the isolates ($n = 214$), representing the full range of resistant norfloxacin MICs, were selected for the simultaneous determinations of ciprofloxacin, gatifloxacin, levofloxacin, and norfloxacin MICs. We found that (i) great MIC variation existed for all four fluoroquinolones, (ii) the ciprofloxacin and levofloxacin MICs of >90% of the fluoroquinolone-resistant isolates were higher than the resistance breakpoints, (iii) ciprofloxacin and levofloxacin MICs were distributed into two distinct groups, (iv) the MICs of two drug pairs (ciprofloxacin and norfloxacin by Kendall's Tau-b test and gatifloxacin and levofloxacin by paired *t* test) were similar with statistical significance but were different from each other, and (v) ~2% of isolates had unprecedented fluoroquinolone MIC relationships. Thus, although the fluoroquinolones can be considered equivalent with regard to clinical susceptibility or resistance, fluoroquinolone MICs differ dramatically for fluoroquinolone-resistant clinical isolates, likely because of differences in drug structure.

Fluoroquinolones, some of the most frequently prescribed antimicrobial agents worldwide, target the bacterial type II topoisomerases gyrase and topoisomerase IV. Type II topoisomerases are essential, ubiquitous enzymes involved in virtually every aspect of DNA metabolism. These enzymes cleave one DNA double helix, pass a second DNA molecule (or a different region of the first DNA molecule) through the break, and religate the broken DNA. Fluoroquinolones increase the longevity of the normally short-lived cleaved DNA-topoisomerase intermediates (reviewed in reference 7). DNA tracking machinery somehow is affected by these intermediates, resulting in multiple subsequent effects, such as chromosome fragmentation, the inhibition of DNA synthesis, and death (reviewed in reference 6).

With regard to susceptibility or resistance defined by CLSI breakpoints (Table 1), the fluoroquinolones appear to exemplify a class effect, such that any decrease in susceptibility (i.e., increased MIC) to one drug means a simultaneous decrease for all (2). The fluoroquinolones, however, vary with regard to pharmacokinetic and pharmacodynamic parameters, including potency (reviewed in reference 24). Additionally, data from defined, isogenic strains of *Escherichia coli* have shown that fluoroquinolone resistance genotypes can affect the MICs of various fluoroquinolones differently (17, 26, 28). For example, regimens of ciprofloxacin (100, 250, 500, or 750 mg twice daily), moxifloxacin (400 mg once daily), and norfloxacin (200 mg twice daily) differ in their ability to eradicate *E. coli* single-mutant (one mutation in the *gyrA* gene of gyrase) and double-mutant (mutations in both *gyrA* and *marR*, which encodes a repressor of the multidrug efflux pump AcrAB) strains in vitro; only 750 mg of ciprofloxacin dosed twice daily eliminated both strains (20). Therefore, although a class effect is apparent with susceptibility breakpoints, one might expect that MICs for fluoroquinolone-resistant isolates vary as a reflection of intrinsic drug differences and how the drugs are affected by various resistance mechanisms.

Here, we analyzed ciprofloxacin, gatifloxacin, levofloxacin, and norfloxacin MICs in fluoroquinolone-resistant *E. coli* clinical isolates. We chose to study these drugs for several reasons: (i) ciprofloxacin and levofloxacin are the most frequently prescribed fluoroquinolones in the United States; (ii) we have hospital-determined susceptibility data for all four drugs (2);

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TABLE 1. Fluoroquinolone MICs for susceptible and resistant *E. coli* clinical isolates

Isolate(s)	MIC (μg/ml)			
	CIP	GAT	LVX	NOR
ATCC 25922 range	0.004–0.016	0.004–0.030	0.008–0.060	0.030–0.120
S isolates avg[a,b]	0.017 ± 0.006	0.020 ± 0.015	0.042 ± 0.039	0.060 ± 0.023
S isolates range[b]	0.004–0.032	0.006–0.064	0.023–0.064	0.023–0.15
CLSI S breakpoint	≤1	≤2	≤2	≤4
CLSI R breakpoint	≥4	≥8	≥8	≥16
R isolates avg[a,c]	85 ± 100	23 ± 31	39 ± 30	232 ± 201
R isolates range[c]	10–500	8–300	8–200	16–1000

[a] Sensitive (S) and resistant (R) were designated for ciprofloxacin (CIP), gatifloxacin (GAT), and levofloxacin (LVX) by the hospitals using the Dade-Behring MicroScan system. NOR, norfloxacin.
[b] MICs for 27 control S isolates were measured by Etest.
[c] MICs for 214 R isolates were measured by agar dilution and Etest methods.

(iii) the accumulation of multiple mutations in the genes encoding gyrase and topoisomerase IV affects ciprofloxacin and norfloxacin MICs ~10-fold more than gatifloxacin and levofloxacin MICs (17); and (iv) the products of the plasmid-borne fluoroquinolone resistance genes *aac(6′)-Ib*-cr and *qepA* affect ciprofloxacin and norfloxacin MICs but not gatifloxacin or levofloxacin MICs (23, 27). In the accompanying study (16), we characterized the known fluoroquinolone resistance genotypes present in these isolates.

MATERIALS AND METHODS

Chemicals and reagents. Mueller-Hinton (MH) agar and broth were purchased from Difco (Sparks, MD). Ciprofloxacin, levofloxacin, and norfloxacin were purchased from Sigma Aldrich (St. Louis, MO), and gatifloxacin was from Bristol-Myers Squibb (New York, NY). Etest strips were purchased from AB Biodisk (Solna, Sweden). API 20E strips were purchased from BioMerieux (Marcy l'Etolle, France).

Clinical isolate collection and culture. *E. coli* isolates in this study originated from two hospitals in Houston, TX. Ben Taub General Hospital (~95% of isolates) is a 578-bed acute-care county hospital serving a largely minority and indigent patient population. The Michael E. DeBakey Veterans Affairs Medical Center (~5% of isolates) has 400 acute- and intermediate-care beds and an additional 150 nursing home care beds. We received clinical isolates on MacConkey or (rarely) blood agar plates, from which we collected all of the colonies using a sterile loop, grew the bacteria overnight in MH broth, and froze the cultures in 1 ml aliquots at −80°C.

Although hospitals already had identified the isolates as *E. coli*, we verified the species for 25 isolates with a broad range of fluoroquinolone MICs and 15 additional isolates with the highest overall MICs using API 20E strips. All isolates were *E. coli*.

Number of isolates. Norfloxacin MICs were determined for 920 clinical isolates of *E. coli*. The automated Dade-Behring MicroScan system at the hospital already had classified ~90% of these as fluoroquinolone resistant, <1% as intermediate, and ~5% as susceptible; ~5% of the isolates did not have accompanying hospital data. The MICs for all isolates determined by the hospitals to be resistant were greater than or equal to the intermediate CLSI breakpoints for all four fluoroquinolones. From the 920 isolates in the pilot study, 214 isolates representing the full range of norfloxacin MICs were chosen for simultaneous MIC measurements for ciprofloxacin, gatifloxacin, levofloxacin, and norfloxacin. To ensure that these isolates were not clonal, we used repetitive sequence-based PCR to determine the percent similarity using the Pearson correlation coefficient. More than 95% of the isolates had genetic similarities of less than 85%, indicating that the isolates were different from each other.

Patient and isolate information. Of the 214 isolates described above, ~71% of isolates were from females and ~29% were from males. The average patient age was 52.3 ± 18.2 years, ranging from 0.75 to 92 years. Bacterial isolates came from the urine (77.6%), blood (6.6%), abdominal exudate (1.6%), wounds (1.6%), kidney (1.2%), sputum (1.2%), nasal cavity (0.8%), and miscellaneous tissues (2.0%). Records for the remaining isolates (7.4%) did not identify a site of origin. Approximately 93% of the isolates came from unique patients; the remaining 16 isolates came from seven repeat patients, with 2 to 5 isolates originating from each.

Fluoroquinolone susceptibility assays. To quantify high-level MICs, we used the agar dilution method, which was modified to include more drug concentrations (1, 10, 20, 30, 40, 50, 100, 200, 300, 400, and 500 μg/ml for ciprofloxacin, gatifloxacin, and levofloxacin and 1, 10, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1,000 μg/ml for norfloxacin). Each clinical isolate, prepared with the direct colony suspension method (18), was applied to MH agar plates in duplicate using a 96-pin microplate replicator (Boekel Scientific, Feasterville, PA). The CLSI American Type Culture Collection (ATCC 25922) *E. coli* reference isolate served as the standard drug-susceptible control for all MIC measurements. Three additional clinical isolates were included as controls in all measurements: ELZ4234 (all drug MICs were <1 μg/ml), ELZ4118 (MICs [μg/ml] were the following: ciprofloxacin, 100; gatifloxacin, 100; levofloxacin, 50; and norfloxacin, 300), and ELZ4251 (MICs [μg/ml] were the following: ciprofloxacin, >500; gatifloxacin, 50; levofloxacin, 200; and norfloxacin, >1,000). The MIC for each clinical isolate was measured at least twice. When the two experiments yielded identical MICs, this number was reported. MICs from independent experiments occasionally varied, but only by one step higher or lower in the dilution series. In this event, the MIC was measured in additional experiments, and the median MIC was reported. When MICs were <1 μg/ml by the agar dilution method, at least two Etest measurements were performed according to the manufacturer's instructions to quantify MICs.

Data storage. All MIC data are housed in a custom-designed, evolving Oracle database (2). An HTML and Java Server Pages-based online user interface enables input and interaction with the database. To minimize potential data entry mistakes, two individuals separately entered the MIC data, which were stored in a preliminary table. Rare inconsistencies in the MIC data were tracked to typographical errors and corrected prior to analysis.

Statistical methods. All parameters were analyzed by paired *t* test (T), with $P \leq 0.001$ considered significant with 99% confidence. When normalized MICs for each pair of the four fluoroquinolones were compared, data also were analyzed by Kendall's Tau-b test, in which positive values represent a significant correlation between drugs, with the association increasing as the correlation coefficient, τ, approaches 1.

RESULTS

Fluoroquinolone MICs in *E. coli* clinical isolates. In a pilot study of 920 *E. coli* clinical isolates (Fig. 1), we found that norfloxacin MICs for fluoroquinolone-resistant isolates ranged from 10 to >1,000 μg/ml (Table 1). Norfloxacin MICs for isolates determined to be susceptible by the hospital ranged from 0.023 to 0.15 μg/ml (Table 1). Approximately 25% of the isolates ($n = 214$), representing the full range of norfloxacin MICs, were selected for simultaneous determinations of ciprofloxacin, gatifloxacin, levofloxacin, and norfloxacin MICs (Fig. 2).

As in the pilot study for norfloxacin, isolates not only were resistant but also the MICs, in some cases for gatifloxacin and

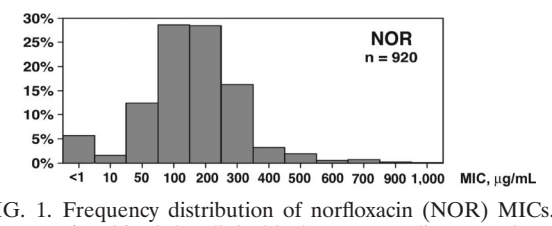

FIG. 1. Frequency distribution of norfloxacin (NOR) MICs. The percentage (*y* axis) of the clinical isolates responding to a given MIC (in micrograms/milliliter; *x* axis) is shown. MIC data from the pilot study using norfloxacin ($n = 920$) are shown.

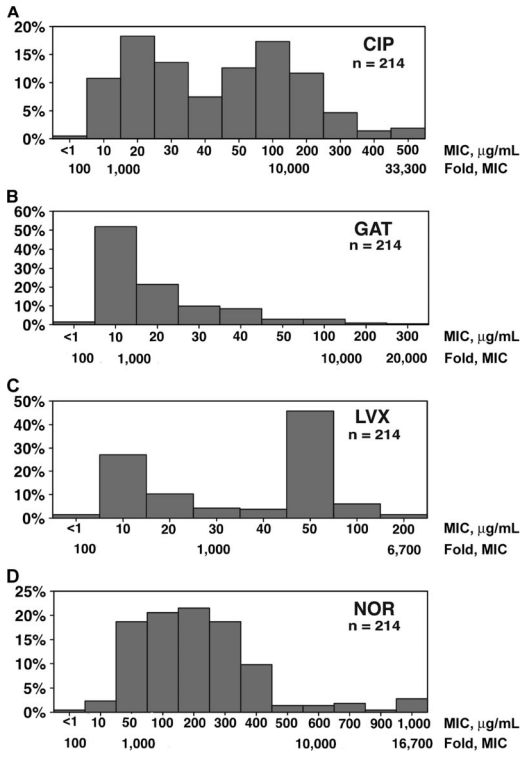

FIG. 2. Frequency distribution of fluoroquinolone MICs. The percentage (*y* axis) of the clinical isolates responding to a given MIC (in micrograms/milliliter; *x* axis) is shown. MICs of ciprofloxacin, CIP (A); gatifloxacin, GAT (B); levofloxacin, LVX (C); and norfloxacin, NOR (D), were determined simultaneously for 214 of the 920 isolates. The MICs were normalized to those for the fluoroquinolone-susceptible clinical isolate ATCC 25922 (0.015 μg/ml ciprofloxacin, 0.015 μg/ml gatifloxacin, 0.03 μg/ml levofloxacin, and 0.06 μg/ml norfloxacin) to give the increase (*n*-fold) in MICs, which are listed below the corresponding MICs along the *x* axis.

in most cases for the other three fluoroquinolones, were very high. Although the fluoroquinolone MICs for susceptible control isolates were similar and did not vary by more than ~3.5-fold (Table 1), there were great differences in the MICs of different drugs for the fluoroquinolone-resistant clinical isolates. The MICs for clinical isolates in general were highest for norfloxacin (reaching ≥1,000 μg/ml); ciprofloxacin, gatifloxacin, and levofloxacin MICs reached as high as 500, 300, and 200 μg/ml, respectively (Table 1). The gatifloxacin MIC for more than half of the isolates was 10 μg/ml (Fig. 2B). Overall, the gatifloxacin and levofloxacin MICs for resistant isolates were lower than ciprofloxacin or norfloxacin MICs (Table 1, Fig. 2). Thus, whereas the fluoroquinolones exhibit a class effect with regard to susceptibility status, great variation is observed for the MICs of different fluoroquinolones in fluoroquinolone-resistant *E. coli* isolates.

Three potential relationships were apparent (Fig. 2). Ciprofloxacin (~40% of isolates) and levofloxacin (~70%) MICs fell into one of two groups that differed by fivefold, 20 and 100 μg/ml for ciprofloxacin and 10 and 50 μg/ml for levofloxacin, indicating two similar groups for the two drugs. The ranges of ciprofloxacin (10 to 200 μg/ml) and norfloxacin (50 to 400 μg/ml) MICs were similar for the vast majority of fluoroquinolone-resistant isolates. Many isolates distributed to a 10-μg/ml peak for both gatifloxacin and levofloxacin MICs.

Normalized fluoroquinolone MICs for *E. coli* clinical isolates. Because susceptible MICs vary by up to 3.5-fold for different fluoroquinolones (Table 1), direct comparisons of MIC data for different fluoroquinolones are difficult. Thus, we normalized the data by determining the increase (*n*-fold) in MIC compared to that for the CLSI standard strain ATCC 25922, which were (within error) 0.015 μg/ml for ciprofloxacin and gatifloxacin, 0.03 μg/ml for levofloxacin, and 0.06 μg/ml for norfloxacin (Table 1). Upon normalization, the clinical isolates exhibited the highest increase (*n*-fold) in resistance to ciprofloxacin (>33,000-fold) (Fig. 2A), indicating that ciprofloxacin MICs might be most affected by resistance mechanisms in the fluoroquinolone-resistant isolates. Normalized norfloxacin MICs increased ~16,700-fold (Fig. 2D). The similarity between resistant isolates with regard to ciprofloxacin (10 to 200 μg/ml) and norfloxacin (50 to 400 μg/ml) MICs was made even more apparent upon normalization; normalized MICs for both drugs ranged from 1,000- to 10,000-fold. Although normalized gatifloxacin and levofloxacin MICs were as high as 20,000-fold and 6,700-fold, respectively, data for 90% of isolates fell below these values. In addition, the normalized MICs of the levofloxacin peaks (~333- and ~1,666-fold) were much lower than those of the ciprofloxacin peaks (~1,333- and ~6,666-fold).

Pairwise fluoroquinolone comparisons. Frequency distribution plots (Fig. 1 and 2) showed how often MICs occurred, but because the MICs were not linked to each isolate, potential relationships between the fluoroquinolones could not be determined in such plots. We therefore examined normalized MICs from each drug in a two-by-two format (Fig. 3). In addition, we analyzed the data statistically using the Kendall's Tau-b test to measure the degree of correlation between the drugs across a range of normalized MICs and the paired *t* test to compare the mean normalized MICs for each drug pair. If the fluoroquinolone MICs are affected similarly, a direct relationship between the normalized MICs of two drugs would be apparent in the graphs, with a correlation coefficient (τ) near 1.0. If the MICs were affected differently, the data points would be expected to fall near one of the graph axes or have a random pattern that is insignificant by Kendall's Tau-b and *t* tests.

Kendall's Tau-b tests uncovered a positive correlation between all drug pairs (Fig. 3), meaning that at least some of the isolates had simultaneous MIC increases for all six drug pairs. These data also confirm the three potential relationships uncovered in Fig. 2. For example, two large bubbles in the panel comparing ciprofloxacin and levofloxacin normalized MICs (Fig. 3) show that the same isolates are responsible for the two distinct groups of ciprofloxacin and levofloxacin MICs in Fig. 2A and C. In addition, a large bubble in the panel comparing gatifloxacin and levofloxacin (Fig. 3) indicates that the same isolates make up the low MIC peaks for the two fluoroquinolones shown in Fig. 2B and C. Ciprofloxacin and norfloxacin were most strongly correlated ($\tau = 0.8$) and exhibited a direct relationship when plotted (Fig. 3). This very high correlation demonstrates that as normalized MICs of ciprofloxacin increase, a simultaneous increase occurs for norfloxacin.

Paired *t* tests revealed a significant relationship ($T = 2.8$; $P = 0.001$) between gatifloxacin and levofloxacin (Fig. 3). These findings indicate that gatifloxacin and levofloxacin are

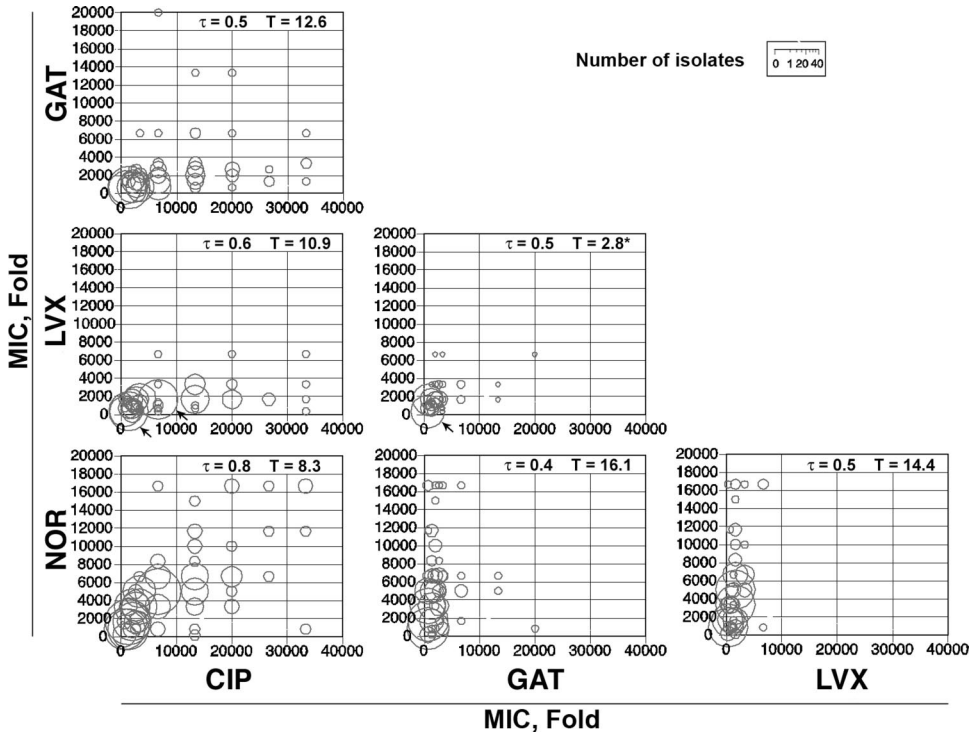

FIG. 3. Pairwise fluoroquinolone comparisons. The *x* and *y* axes for each panel show the normalized MICs of each possible pair of the four fluoroquinolones. The size of each bubble indicates the number of isolates for each (*x*, *y*) point; the key is shown. Kendall's Tau-b test (τ) correlation coefficients and paired *t* test (T) results are given for each panel at the top right. The degree of correlation between each drug pair increases as τ approaches 1.0. The asterisk indicates significance ($P < 0.001$) by the paired *t* test. The two arrows in the ciprofloxacin (CIP) and levofloxacin (LVX) panel denote the isolates represented by the two large bubbles that are seen as peaks in Fig. 2A and C. The arrow in the CIP and gatifloxacin (GAT) panel denotes the isolates, represented by the large bubble, that also form a single low MIC peak, which is shown in Fig. 2A and B.

affected to the same extent by resistance mechanisms in many isolates. No other significant relationship was uncovered by paired *t* tests between any other drug pair.

Comparisons of normalized MICs of three fluoroquinolones. The pairwise comparisons raised important additional questions. Would high normalized ciprofloxacin and norfloxacin MICs correlate with each other but not with gatifloxacin and levofloxacin normalized MICs, as one might expect based upon differences in drug activity and how resistance mechanisms affect the different fluoroquinolone MICs? Were the isolates that were responsible for the relationship between ciprofloxacin and levofloxacin the same as those responsible for gatifloxacin and levofloxacin? Did other additional relationships exist among the drugs? To answer these questions, we compared normalized MIC data from three drugs simultaneously.

When data for either gatifloxacin or levofloxacin, but not norfloxacin, was compared with data for any drug pair, <5% of the isolates indeed had intermediate normalized MICs for all three drugs (data not shown). Thus, these clinical isolates all have similar intermediate resistance to ciprofloxacin, gatifloxacin, levofloxacin, and norfloxacin and thus exhibit a class effect. Although a correlation existed between all pairs of fluoroquinolones by Kendall's Tau-b tests, the remaining isolates (~95%) generally did not exhibit a class effect-like relationship for any three drugs. For example, isolates generally did not have high normalized MICs for any three drugs simultaneously. In general, as predicted, ciprofloxacin and norfloxacin data correlated with each other, as did gatifloxacin and levofloxacin data, but the two drug pairs did not correlate with each other.

These three-way analyses uncovered three additional unprecedented, infrequent phenotypes (Table 2). The normalized gatifloxacin and levofloxacin MICs for ELZ4004 and ELZ4686 were high, but the normalized ciprofloxacin and norfloxacin MICs were low. This phenotype was the opposite of what was expected (as seen in Fig. 2) for the known mechanisms [i.e., topoisomerase mutations, Aac(6′)-Ib-cr, and QepA] predicting high ciprofloxacin and norfloxacin MICs but low gatifloxacin and levofloxacin MICs. The normalized norfloxacin MICs for ELZ4132 were high, but the values for other drugs were low. Normalized gatifloxacin MICs for ELZ4601 were low, but the normalized MICs of the other three fluoroquinolones were high. The fluoroquinolone MICs for these isolates were verified by additional repeated agar dilution MIC determinations. As discussed in the accompanying study, these unusual isolates had, at most, four mutations in the genes encoding gyrase and topoisomerase IV and increased levels of the multidrug efflux pump AcrAB, which do not cause such MIC phenotypes in defined, isogenic strains (16).

To determine both the frequency of the unusual phenotypes and to search for any other such phenotype, we screened 675

TABLE 2. *E. coli* clinical isolates with unprecedented fluoroquinolone resistance phenotypes

Isolate(s)	Phenotype[c]				Frequency (*n*)	
	Ciprofloxacin	Gatifloxacin	Levofloxacin	Norfloxacin	Set 1[a] (*n* = 214)	Set 2[b] (*n* = 675)
ELZ4004, ELZ4686	⇓	⇑	⇑	⇓	0.9 (2)	0.5 (3)
ELZ4132	⇓	⇓	⇓	⇑	0.5 (1)	0.7 (5)
ELZ4601	⇑	⇓	⇑	⇑	0.5 (1)	0.3 (2)

[a] Three MIC phenotypes, which no combination of known resistance genotypes is known to cause, were uncovered in 4 of 214 isolates.
[b] Data from screens of 675 additional isolates for the three phenotypes uncovered 10 additional isolates.
[c] Up and down arrows indicate high (>20,000-fold for ciprofloxacin, >15,000-fold for gatifloxacin, >5,000-fold for levofloxacin, and >15,000-fold for norfloxacin) or low (<3,000-fold for ciprofloxacin, <2,000-fold for gatifloxacin, <1,500-fold for levofloxacin, and <2,500-fold for norfloxacin) normalized fluoroquinolone MICs relative to those of the total population of isolates.

additional fluoroquinolone-resistant isolates with distinguishing fluoroquinolone concentrations (300 μg/ml ciprofloxacin, 100 μg/ml gatifloxacin, 100 μg/ml levofloxacin, and 500 μg/ml norfloxacin). In each experiment, ELZ4004, ELZ4132, ELZ4601, and ELZ4686 were included as controls. Thirty-six isolates were highly resistant to at least one of the four fluoroquinolones, and MICs were determined for these isolates by the agar dilution method. Three isolates (ELZ4670, ELZ4742, and ELZ4800) had a phenotype like those of ELZ4004 and ELZ4686; five isolates (ELZ4330, ELZ4347, ELZ4457, ELZ4595, and ELZ4729) had the same resistance phenotype as that of ELZ4132; and two isolates (ELZ4735 and ELZ4797) had a phenotype like that of ELZ4601 (Table 2). No additional new phenotypes were uncovered; however, ~1.5% of the 675 screened isolates had the same three unexpected phenotypes as those found above (Table 2). This frequency was nearly identical to that of the initial experiment (1.9%). Thus, a small fraction of *E. coli* clinical isolates appear to contain resistance mechanisms that affect fluoroquinolone MICs differently from any known resistance genotypes.

DISCUSSION

A structural basis for fluoroquinolone relationships. The correlation between ciprofloxacin and norfloxacin MICs and the significant relationship between gatifloxacin and levofloxacin MICs likely result from similarities in the drug structures of the two pairs. Ciprofloxacin and norfloxacin lack C-8 substitutions on their fluorinated quinoloic acid cores and differ only at position N1, where ciprofloxacin has a cyclopropane ring and norfloxacin has an ethyl group. In contrast to ciprofloxacin and norfloxacin, gatifloxacin and levofloxacin both have oxygenated C-8 substitutions. In vitro experiments have shown that purified gyrase is somewhat more sensitive to fluoroquinolones, like gatifloxacin and levofloxacin, with C-8 substitutions (3, 11). Fluoroquinolones with a C-8-methoxy group have increased potency against *E. coli*, *Staphylococcus aureus*, and mycobacterial strains with topoisomerase mutations compared to wild-type, parental strains (4, 13, 30). The presence of a C-8-methoxy substitution also lowers the concentration of antibiotic required to block the growth of first-step mutants, or the mutant prevention concentration, in these same bacterial species (5, 29). Thus, biochemical and microbiological data likely explain why the two drug pairs correlated and why similar correlations among any three drugs were seen only in a subset (<5%) of isolates.

Known resistance genotypes also can account for the relationships between ciprofloxacin and norfloxacin and between gatifloxacin and levofloxacin. As presented in the introduction, MICs for strains containing multiple mutations in the topoisomerase genes are ~10-fold higher for ciprofloxacin and norfloxacin than gatifloxacin and levofloxacin (17). In addition, ciprofloxacin and norfloxacin, but not gatifloxacin or levofloxacin, are substrates of the plasmid-encoded acetyltransferase Aac(6′)-Ib-cr (16) and efflux pump QepA (21, 27). Given these differences, the source of the two distinct peaks of ciprofloxacin and levofloxacin MICs (Fig. 2A and C) is not clear. The isolates in the higher ciprofloxacin and levofloxacin MIC peaks may have accumulated more resistance-associated genetic alterations or plasmid-borne genes than those in the lower peaks. In the accompanying report (16), we find that this indeed was the case.

Selection of high fluoroquinolone MICs for clinical isolates. CLSI resistance breakpoint MICs range from 2 to 16 μg/ml, depending on the fluoroquinolone (Table 1) (19); however, the measured MICs for fluoroquinolone-resistant *E. coli* clinical isolates originating largely from Ben Taub General Hospital are much higher than this, reported previously as being up to 8,000-fold (10) and, now, even 33,000-fold higher. Studies of isolates from a broader geographic distribution will determine whether such high MICs are widespread. During dosing, drug concentrations range from subtherapeutic to therapeutic over time. Sub-MIC fluoroquinolone concentrations increase the mutation frequency in *E. coli* and are known to increase fluoroquinolone MICs slightly for *Mycobacterium fortuitum* and *S. aureus* in vitro (8, 9). During treatment, both the bacteria causing disease and bystander bacteria not causing disease are exposed to sub-MIC doses of fluoroquinolones. These bacteria may acquire initial mutations, allowing them to survive in order to accumulate additional mutations. Levofloxacin and norfloxacin can reach peak concentrations of >400 μg/ml in the bladder, and ciprofloxacin can accumulate to 2 mg of drug per gram of feces in the gastrointestinal tract (1). Thus, higher fluoroquinolone concentrations can select for additional genetic alterations that account for the high observed MICs. It is possible that the hospitals from which these isolates originated routinely used dosing regimens that included high doses, which would enrich only those isolates with multiple resistance-associated mutations and result in populations of isolates for which the MICs were high (20).

Exposure to nonfluoroquinolone antibiotics could have selected for genetic alterations involving multidrug resistance mechanisms. The multidrug efflux pump AcrAB and the plasmids harboring fluoroquinolone resistance genes have been

associated with multidrug resistance. Thus, some of the isolates could have acquired fluoroquinolone resistance as an unintended side effect of treatment with other antibiotics. In the accompanying article (16), we characterize which of the known resistance mechanisms are present in these isolates.

Any of these potential mechanisms may select for a mutation that confers a fitness advantage to the isolates. For example, defined, fluoroquinolone-resistant strains of *Campylobacter jejuni* containing *gyrA* mutations were able to outcompete susceptible, isogenic strains in an animal model (14). The selection mechanisms also could have selected for the overgrowth of mutator isolates with deficiencies in DNA damage repair pathways (15, 22). Multiple rounds of in vitro ofloxacin selection with the *E. coli dnaQ49* mutator strain, for example, resulted in ofloxacin MICs of 3,000 μg/ml (25). High mutation rates, which allow for the increased presence of rare, resistance-associated mutations, correlated with fluoroquinolone resistance in fluctuation tests of 54 *E. coli* clinical isolates from urinary tract infections (12).

Taken together, these data extended previous findings of the effects of drug structure on fluoroquinolone MICs. They also demonstrated that ciprofloxacin, gatifloxacin, levofloxacin, and norfloxacin MICs for fluoroquinolone-resistant *E. coli* clinical isolates can be extremely high. In addition, a small percentage of these isolates may contain novel resistance genotypes. To determine to what extent known resistance mechanisms are present in all of the resistant isolates, we characterize these mechanisms in the companion study (16).

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